Microbiological Hydroxylation. Part XV.[1] Hydroxylation in the Terminal Rings of Mono- and Di-oxygenated 5α-Androstanes with the Fungus *Daedalea rufescens*

By **Alan M. Bell, Sir Ewart R. H. Jones, G. Denis Meakins,* John O. Miners,** and **Anthony Pendlebury,** Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY

Mono- and di-ketones and keto-alcohols derived from 5α-androstane have been incubated with the fungus *Daedalea rufescens*, a Basidiomycete species not previously reported as a steroid hydroxylator. All but one of the dioxygenated substrates, and the monoketones with the keto-group in a central ring, are hydroxylated fairly rapidly. The cleanest hydroxylations occur with 7-oxygenated androstanes and lead to the 3β,16β-dihydroxy-derivatives.

Incubation of 3,3-ethylenedioxy-5α-androstan-7-one (in which hydroxylation is accompanied by reduction of the 7-keto-group) followed by hydrolysis of the product gives 7α,16β-dihydroxy-5α-androstan-3-one in 57% yield.

In the preceding Part [1] the hydroxylation of certain oxygenated 5α-androstanes with *Wojnowicia graminis* and *Ophiobolus herpotrichus*, two Ascomycete fungi, was reported; the main feature was the ability of these micro-organisms to hydroxylate the terminal rings of some substrates. Efficient hydroxylation occurred only with dioxygenated androstanes, a result which imposes a severe limitation on the usefulness of these fungi. In searching for micro-organisms which are less demanding in their substrate requirements, we screened a number of Basidiomycete species. Of these *Daedalea rufescens* (not previously reported as a steroid hydroxylator [2]) appeared one of the most promising, and was therefore selected for systematic investigation.

Table 1 summarises the microbiological results obtained by incubating some monoketones, diketones, and derived mono-acetals, and keto-alcohols derived from 5α-androstane with vegetative cell cultures of *Daedalea rufescens*. Table 2 lists the n.m.r. spectra of the steroids, substrates, and products, involved here for which spectroscopic data have not appeared in earlier publications: the arabic serial number sequence of steroids discussed earlier [3] is used in this Table, which contains steroids nos. 732—752. The structures of new compounds follow, as usual,[3] from a combination of spectrometric and chemical methods. For new compounds the n.m.r. signals appear in Table 2, and the other information required for their characterisation is given in Table 3. As with the earlier paper,[1] and for reasons discussed there, the whole of the Experimental section [4] has been deposited as Supplementary Publication No. SUP 21218 (10 pp., 1 microfiche).†

A striking feature is the contrast between the slow hydroxylation of the monoketones having the carbonyl group in a terminal ring and the much faster substitution of the isomers with the group in a central ring. While the latter (the 6-, 7-, and 11-ketones) behave similarly, in that hydroxylation (possibly followed by oxidation of a new hydroxy-group) occurs in both rings A and D, the processes differ markedly in specificity: the 6- and 11-ketones give several products whereas with the 7-ketone

† For details of Supplementary Publications, see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1974, Index issue.

[1] Part XIV, V. E. M. Chambers, Sir Ewart R. H. Jones, G. D. Meakins, J. O. Miners, and A. L. Wilkins, *J.C.S. Perkin I*, 1975, 55.

[2] W. Charney and H. L. Herzog, 'Microbial Transformations of Steroids,' Academic Press, New York, 1967.

[3] A. M. Bell, P. C. Cherry, I. M. Clark, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and P. D. Woodgate, *J.C.S. Perkin I*, 1972, 2081.

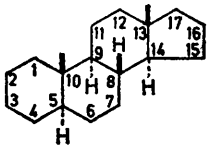
[4] Full details of the microbiological and chemical operations are recorded by A. Pendlebury, D.Phil. Thesis, Oxford, 1972.

remarkably selective 3β,16β-dihydroxylation is accompanied by reduction of the carbonyl function, mainly to the 7α-alcohol.

All the dioxygenated androstanes apart from the 3-oxo-7-acetal are hydroxylated fairly rapidly. The cleanest processes (again leading to 3,16-disubstitution or to monosubstitution if one of these positions is blocked) occur with substrates having a 7-oxo- or a 7α-hydroxy-group; the 7β-hydroxy-compound and the 7-acetals are utilised less efficiently. The tendency of *D. rufescens* to hydroxylate positions 3 and 16 appears to operate, but less clearly, with 11-oxo-substrates. Thus 15-hydroxylation of the 3,11-dione and of the 3β-hydroxy-11-ketone competes more evenly with 16-hydroxylation, and 3-substitution does not dominate the situation with the 11,17-diketone.

TABLE 1

Hydroxylations with *Daedalea rufescens*



5α-Androstane

The substrates, all derivatives of 5α-androstane are indicated by trivial names, *e.g.* 3β-OH–17-CO represents 3β-hydroxy-5α-androstan-17-one. In the 'Products' columns those oxygen functions introducd during the incubation are in bold type, and n.i. indicates that no product was isolated. The substrates were introduced as solutions in ethanol and incubated for the times (usually 4 or 6 days) specified in the Experimental section. The yields are calculated after making allowance for recovered starting material.

Substrate	Substrate recovered (%)	Main hydroxylation product(s)		Other products	
3-CO	90	n.i.			
6-CO	21	**3**β,6α, **16**β-$(OH)_3$	16%	**3**β,6α-$(OH)_2$–**16**-CO	5%
				3β, **16**β-$(OH)_2$	4
				3β, **15**β-$(OH)_2$	2
7-CO	20	**3**β,7α, **16**β-$(OH)_3$	41		
		3β,7β, **16**β-$(OH)_2$	16		
11-CO	10	**3**β, **14**α,**16**β-$(OH)_3$	12	**3**β-OH– **16**-CO	7
				3β, **16**α-$(OH)_2$	5
				3β, **16**β-$(OH)_2$	3
				3β, **15**β-$(OH)_2$	1
				2β, **16**β-$(OH)_2$	0·5
16-CO	58	Complex mixture, n.i.			
17-CO	50	Complex mixture, n.i.			
3,6-$(CO)_2$	9	3β, **16**β-$(OH)_2$	39		
		3β,6α, **16**β-$(OH)_3$	25		
		3β, **15**β-$(OH)_2$	12		
3,7-$(CO)_2$	3	3β,7α, **16**β-$(OH)_2$	33	3β,7β, **16**β-$(OH)_3$	13
				3β -OH	5
				3β,7α-$(OH)_2$–**16**-CO	3
				3β,7α -$(OH)_2$	2·5
				3β,7β -$(OH)_2$	2·5
7,7-[O,O]-3-CO	50	3β,7α, **16**β-$(OH)_3$	30		
3,3-[O,O]-7-CO	19	7α, **16**β-$(OH)_2$	66	3-CO–7α, **16**β-$(OH)_2$	16
				3β,7α, **16**β-$(OH)_2$	7
7α-OH–3-CO	28	**16**β-OH	53		
		3β, **16**β-$(OH)_2$	28		
7β-OH–3-CO	33	3β -OH	18		
3,11-$(CO)_2$	5	3β, **16**β-$(OH)_2$	28		
		3β, **15**β-$(OH)_2$	15		
3β-OH–11-CO	0	**16**β-OH	17	**15**β-OH	8
				14α,**16**β-$(OH)_2$	5
				16-CO	3·5
3,17-$(CO)_2$	0	3β,7β, 17β-$(OH)_3$	24	3β, **11**α,17β-$(OH)_3$	13
				3β, **6**α 17β-$(OH)_3$	11
17β-OH–3-CO	3	Complex mixture, n.i.			
3β-OH–17-CO	15	Complex mixture, n.i.			
7,11-$(CO)_2$	0	3β,7α, **16**β-$(OH)_3$	42		
7,16-$(CO)_2$	0	3β,7α -$(OH)_2$	39		
7,17-$(CO)_2$	0	3β,7α, 17β-$(OH)_3$	32	3-CO–7α, 17β-$(OH)_2$	5
		3β,7β, 17β-$(OH)_3$	16		
11,17-$(CO)_2$	0			3β,7β, 17β-$(OH)_3$	9
				3β, 17β-$(OH)_2$	7

The results can be interpreted along lines similar to those adumbrated in the preceding Part [1] for the fungi *W. graminis* and *O. herpotrichus*. Thus *D. rufescens* has a propensity for attacking certain positions, chiefly 3, 7,

TABLE 2

N.m.r. signals

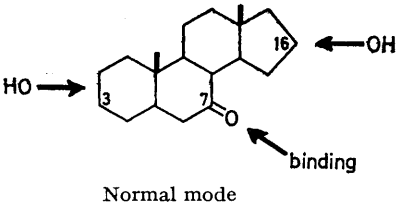
The results, presented in the form used earlier,[a] were obtained by examining solutions in $CDCl_3$ at 100 MHz. The weak signals recorded with saturated solutions of some relatively insoluble triols are not given: each of these triols is followed by an entry for the corresponding acetate.

No.	Compound		τ_3	τ_2 (calc.)	>CH–OR and other characteristic signals		
732	7,7-Ethylenedioxy-5α-androstan-3-one	19	8·95	8·95	(Acetal)	6·03	s
		18	9·27	9·26			
733	5α-Androstane-7,16-dione	19	8·92	8·90			
		18	9·13	9·13			
734	5α-Androstane-2,11,16-trione	19	8·96	8·99			
		18	9·16	9·16			
735	5α-Androstane-3,11,15-trione	19	8·77	8·75			
		18	9·21	9·22			
736	5α-Androst-14-ene-3,11,16-trione	19	8·65	8·63	H-15	3·99	s
		18	8·75	8·75			
737	7,7-Ethylenedioxy-5α-androstan-3β-ol	19	9·16	9·16	H-3	6·42	7 (10, 10, 5, 5)
		18	9·30	9·29	(Acetal)	6·03	s
738	14α-Hydroxy-5α-androstane-3,11,16-trione	19	8·75	8·72			
		18	9·00	9·01			
739	5α-Androstane-7α,16β-diol	19	9·18	9·20	H-7	6·16	m (7)
		18	9·03	9·05	H-16	5·55	m (16)
740	2β,16β-Dihydroxy-5α-androstane-11-one	19	8·74	8·72	H-2	5·92	m (8)
		18	9·09	9·08	H-16	5·53	m (15)
741	3β,6α-Dihydroxy-5α-androstane-16-one	19	9·12	9·12	H-3 }	6·5	m (23)
		18	9·14	9·13	H-6 }		
742	3β,15β-Dihydroxy-5α-androstan-6-one	19	9·21	9.22	H-3	6·44	7 (10, 10, 5, 5)
		18	8·99	9·00	H-15	5·75	t (6)
743	3β,15β-Dihydroxy-5α-androstan-11-one	19	8·94	8·95	H-3	6·43	m (24)
		18	9·05	9·05	H-15	5·59	t (6)
744	3,3-Ethylenedioxy-5α-androstane-7α,16β-diol	19	9·16	9·17	H-7	6·15	m (7)
					H-16	5·54	m (16)
		18	9·05	9·06	(Acetal)	6·05	s
745	7α,17β-Dihydroxy-5α-androstan-3-one	19	8·99	8·98	H-7	6·14	m (7)
		18	9·24	9·23	H-17	6·32	t (8)
746	3β,7α,16β-Triacetoxy-5α-androstane	19	9·14	9·14	H-3	5·27	m (22)
					H-7	5·07	m (8)
		18	9·12	9·13	H-16	4·83	m (18)
747	3β,7β,16β-Triacetoxy-5α-androstane[b]	19	9·11	9·11	H-3	5·27	m (22)
					H-7	5·40	m (19)
		18	9·09	9·09	H-16	4·83	m (18)
748	[3β,7α,16β-Trihydroxy-5α-androstan-11-one]						
749	3β,7α,16β-Triacetoxy-5α-androstan-11-one	19	8·95	8·92	H-3	5·30	7 (10, 10, 5, 5)
					H-7	5·00	m (6)
		18	9·16	9·14	H-16	4·77	m (15)
750	3β,7β,17β-Trihydroxy-5α-androstan-11-one	19	8·92	8·90	H-3 }	6·4	m (22)
					H-7 }		
		18	9·28	9·30	H-17	6·17	t (8)
751	[3β,14α,16β-Trihydroxy-5α-androstan-11-one]						
752	3β,16β-Diacetoxy-14α-hydroxy-5α-androstan-11-one	19	8·96	8·93	H-3	5·34	7 (10, 10, 5, 5)
		18	9·03	9·04	H-16	4·61	m (20)

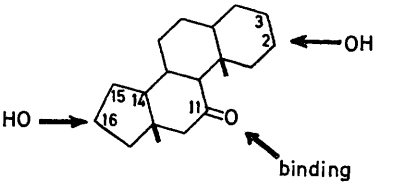
[a] Ref. 3. [b] Not fully characterised.

and 16, of suitable substrates. Three sites, so disposed as to correspond closely to positions 3, 7, and 16 of the steroid nucleus, are thought to be present on the enzyme surface (Scheme). While each site is regarded as being capable of binding to a suitable oxygenated group of the substrate and of hydroxylating a carbon centre of the steroid molecule which becomes close to it in the enzyme–steroid complex, the sites are presumed to differ in their main tendencies, *viz.*, binding at the central site and hydroxylation at the terminal sites. The orientation on the enzyme surface taken up by substrates having a 7-oxo- or a 7α-hydroxy-group is then peculiarly suited to clean hydroxylation. With 11-ketones, binding in the reverse mode[5] does not produce so close a fit between particular carbon centres and the hydroxylating sites,

[5] V. E. M. Chambers, W. A. Denny, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, G. D. Meakins, and J. Pragnell, *J.C.S. Perkin I*, 1973, 1500.



Normal mode



Reverse mode

SCHEME Model for hydroxylations with *D. rufescens*

and substitution occurs at several positions. The lack of reactivity of the terminal ring monoketones is again considered to indicate that binding of the oxo-groups to the terminal sites is weak, or that the complex produced difficult to obtain by normal chemical methods, the efficient hydroxylations of the 7-oxo-3-acetal and the 7α-hydroxy-3-ketone are noteworthy. These (followed in the former case by hydrolysis of the acetal group) lead to

TABLE 3

Characterisation of new compounds

Compound	M.p. (°C) *	$[\alpha]_D$ † (conc.)		Analytical figures (%) C	H
7,7-Ethylenedioxy-5α-androstan-3-one	150—152	−6°	Found	75·8	9·7
		(1·0)	$C_{21}H_{32}O_3$ req.	75·9	9·7
5α-Androstane-7,16-dione	174—175	−232	Found	79·0	9·7
		(1·0)	$C_{19}H_{28}O_2$ req.	79·1	9·8
5α-Androstane-2,11,16-trione	222—224 ‡	−105	Found	75·5	8·7
		(0·1)	$C_{19}H_{26}O_3$ req.	75·5	8·7
5α-Androstane-3,11,15-trione	152—154 §	+85	Found	75·8	8·6
		(0·3)	$C_{19}H_{26}O_3$ req.	75·5	8·7
5α-Androst-14-ene-3,11,16-trione	120—122	+143	Found	75·8	8·3
		(0·1)	$C_{19}H_{24}O_3$ req.	76·0	8·05
14α-Hydroxy-5α-androstane-3,11,16-trione	281—283	−81 ¶	Found	71·6	8·1
		(0·45)	$C_{19}H_{26}O_4$ req.	71·7	8·2
5α-Androstane-7α,16β-diol	174—175·5	−28	Found	77·8	10·7
		(1·0)	$C_{19}H_{32}O_2$ req.	78·0	11·0
2β,16β-Dihydroxy-5α-androstan-11-one	185—187	+50	Found	74·2	9·9
		(0·2)	$C_{19}H_{30}O_3$ req.	74·5	9·9
3β,6α-Dihydroxy-5α-androstan-16-one	235—238	−127	Found	74·3	9·7
		(0·6)	$C_{19}H_{30}O_3$ req.	74·5	9·9
3β,15β-Dihydroxy-5α-androstan-6-one	228—229	−68	Found	74·5	9·8
		(0·9)	$C_{19}H_{30}O_3$ req.	74·5	9·9
3β,15β-Dihydroxy-5α-androstan-11-one	243—244	+10	Found	74·6	9·6
		(1·0)	$C_{19}H_{30}O_3$ req.	74·5	9·9
3,3-Ethylenedioxy-5α-androstane-7α,16β-diol	205—206·5	−23	Found	71·7	9·6
		(1·0)	$C_{21}H_{34}O_4$ req.	71·95	9·8
3β,7α,16β-Triacetoxy-5α-androstane	151—153	−61	Found	69·3	8·7
		(1·0)	$C_{25}H_{38}O_6$ req.	69·1	8·8
3β,7α,16β-Trihydroxy-5α-androstan-11-one	283—285	+21	Found	70·8	9·4
		(1·0)	$C_{19}H_{30}O_4$ req.	70·8	9·4
3β,7α,16β-Triacetoxy-5α-androstan-11-one	244—246	−19	Found	67·1	8·2
		(1·0)	$C_{25}H_{36}O_7$ req.	66·9	8·1
3β,7β,17β-Trihydroxy-5α-androstan-11-one	235—237 ‖	+58 **	Found	67·3	9·6
		(0·5)	$C_{19}H_{30}O_4$ req.	67·0	9·5
3β,14α,16β-Trihydroxy-5α-androstan-11-one	269·5—270 ††	+49	Found	67·1	9·3
		(1·0)	$C_{19}H_{30}O_4$ req.	67·0	9·5
3β,16β-Diacetoxy-14α-hydroxy-5α-androstan-11-one	224—226	+43	Found	68·0	8·4
		(0·5)	$C_{23}H_{34}O_6$ req.	68·0	8·4

* From acetone–hexane unless otherwise specified. † Rotations determined with $CHCl_3$ as solvent unless otherwise indicated. ‡ From acetone. § From EtOH. ¶ Rotation determined with dioxan as solvent. ‖ From $CHCl_3$–MeOH. ** Rotation determined with EtOH as solvent. †† From MeOH.

does not bring other steroid centres close enough to the hydroxylating sites. Although this pictorial approach is not sufficiently precise for a detailed treatment of the results, it serves as a useful model in planning preparative sequences involving micro-organisms.

In connection with the synthesis of steroids which are the 3-oxo-7α,16β-diol and thence, by Huang-Minlon reduction, to 5α-androstane-7α,16β-diol.

We thank the S.R.C. for a studentship (to A. P.) and a fellowship (to J. O. M.), and Glaxo Research Ltd., for a grant and gifts of chemicals.

[4/1953 *Received, 24th September,* 1974]